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ARTICLE

Dietary Supplementation with α-Amylase Inhibitor Wheat Albumin to High-Fat Diet-Induced Insulin-Resistant Rats Is Associated with Increased Expression of Genes Related to Fatty Acid Synthesis in Adipose Tissue

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It is well-known that insulin resistance induces lipid abnormalities by decreasing insulin actions in adipose tissue. This study examined the effects of inhibiting postprandial hyperglycemia/hyperinsulinemia, using the α-amylase inhibitor wheat albumin (WA), on the expression of genes related to fatty acid metabolism in the adipose tissue of high-fat diet-induced insulin-resistant rats. Postprandial glucose and insulin levels were significantly lower after oral starch loading with WA than with inactivated WA in insulin-resistant rats. In addition, the increases in the plasma triacylglycerol and insulin levels by feeding insulin-resistant rats a control diet were inhibited by WA supplementation. Supplementation with WA increased the mRNA levels of not only fatty acid synthase (FAS) and acyl-CoA carboxylase (ACC) but also their transcriptional factors such as carbohydrate response element-binding protein (ChREBP) and sterol regulatory element binding protein (SREBP)1 in the mesenteric adipose tissue of the insulin-resistant rats. In addition, supplementation with WA tended to increase the protein expression levels of FAS and ACCs. These results suggest that reductions in the plasma triacylglycerol and insulin levels by inhibiting hyperglycemia/hyperinsulinemia with the α-amylase inhibitor WA in high-fat diet-induced insulin-resistant rats are associated with increased expression of genes related to fatty acid synthesis and their transcriptional factors in adipose tissue.

KEYWORDS: α-Amylase inhibitor; wheat albumin; adipose tissue; insulin resistance; fatty acid synthesis

INTRODUCTION

It is well-known that insulin secretion caused by postprandial hyperglycemia not only reduces plasma glucose concentrations but also induces fatty acid synthesis in the liver, adipose tissue, and other peripheral tissues. Recently, many studies have shown that the actions of insulin in these tissues are diminished by feeding rats a high-fat diet (*1*). This impairment of insulin actions is known as insulin resistance and is one of the major causes of the development of diabetes. Indeed, fatty acid synthesis in animal models with insulin resistance is known to be lower than that in normal animals (*2*). In addition, treatment with one of the molecules known to induce insulin resistance, interleukin (IL)-6, reduces the mRNA level of fatty acid synthase (FAS), which is a rate-controlling enzyme for fatty acid synthesis (*3*). Furthermore, treatment of 3T3-L1 adipocytes with rosiglitazone (*3*) or troglitazone (*4*), which are thiazolidine derivatives and ameliorate insulin resistance by activating peroxisome proliferator-activated receptor (PPAR)γ, induces FAS mRNA expression. Moreover, amelioration of insulin resistance by fructo-oligosaccharides (*5*) or pioglitazone (*6*), another thiazolidine derivative, induces fatty acid synthesis in rats. Furthermore, human studies have revealed not only that decreased FAS mRNA levels in adipose tissue are associated with insulin resistance but also that amelioration of insulin resistance by pioglitazone increases the gene expression in adipose tissue (*7*). When it is taken into account that insulin-resistant adipose tissue has lower activity for glucose incorporation, the results of these studies indicate that insulin resistance leads to decreased fatty acid synthesis accompanied by reduced glucose incorporation.

Recently, various food components and drugs that decrease postprandial hyperglycemia by inhibiting α-amylase secreted from the pancreas or α-glucosidases in the small intestine have been developed (*8*−*10*). Wheat albumin (WA), which is extracted from wheat, has been reported to be an inhibitor of pancreatic α-amylase and to decrease postprandial hyperglycemia (*10*−*13*).

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The specific α-amylase-inhibiting proteins in WA have already been identified as 0.19, 0.28, 0.36, and 0.53 fractions by gel electrophoresis (*14*). The 0.19 fraction was found to exert the predominant α-amylase inhibitory activity in WA (*11*, *12*). Purified WA including these fractions has greater inhibitory effects on α-amylase activity than other types of α-amylase inhibitors, such as white beans (*11*). It is expected that WA will have greater effects on reducing postprandial glucose levels and insulin secretion at an earlier time than other inhibitors, because WA can be given to patients in high concentrations with less incidence of hypoglycemia and digestive symptoms, such as diarrhea (*10*), since it is largely digested in the small intestine. Recent studies have demonstrated that treatment with the α-glucosidase inhibitor acarbose ameliorates the resistance of insulin-regulated GLUT4 trafficking in insulin-resistant animal model Zucker fatty rats (*15*). In addition, a human study has demonstrated that treatment of NIDDM patients with acarbose improves insulin resistance (*16*). These observations indicate that inhibition of postprandial hyperglycemia may ameliorate insulin resistance by altering fatty acid metabolism following glucose incorporation. However, it is unclear whether inhibition of postprandial hyperglycemia by drugs or food components including WA alters the expression of genes related to lipid metabolism in the adipose tissue of insulin-resistant animals.

In this study, we examined the acute effects of a single dose of WA on blood glucose concentrations and insulin secretion after starch loading, as well as the chronic effects of long-term WA treatment in a dietary mixture on glycemic control and lipid metabolism in the blood and adipose tissue of insulin-resistant rats.

MATERIALS AND METHODS

Animals. Sprague−Dawley (SD) male rats were obtained from SLC Japan Inc. (Tokyo, Japan). They were housed in a room controlled for temperature (22 ± 3 °C), humidity (55 ± 15%), and light (diurnal time from 7:00 a.m. to 7:00 p.m.). The rats were randomly assigned to individual groups on the basis of their body weight and the results of an oral glucose tolerance test (OGTT) (2 g/kg of body weight). Five rats had free access to a standard laboratory chow (MF; Oriental Yeast, Tokyo, Japan), and 15 rats had free access to a high-fat diet [as percentages of the total energy: 32.1% safflower oil [high oleic type provided by Nisshin Oillio (Yokohama, Japan)], 32.1% lard, 14.3% casein, 14.3% sucrose, 7.1% α-cornstarch; supplemented with vitamins and minerals based on AIN-93]. After 11 weeks, we confirmed by an OGTT that animals fed the high-fat diet developed modulate insulin resistance, as described previously (*17*).

Single-Dose Starch Loading with WA in Adult Rats with Insulin Resistance. After 1 week of continuous feeding of the high-fat diet after the OGTT, the rats exhibiting insulin resistance were randomly assigned to two groups (six or eight rats per group) on the basis of their body weight and the results of the OGTT at 11 weeks. The rats were fasted for 14 h before the experiments.

One group of animals with insulin resistance (n = 8) was administered a single oral dose of WA (400 mg/kg of body weight) dissolved in 5% (w/v) starch solution. The other group of insulin-resistant rats (n = 6) and the control rats fed a laboratory chow diet (n = 5) were administered heat-inactivated WA (400 mg/kg of body weight) mixed in 5% (w/v) starch solution. Purified WA was prepared as previously described (*12*). WA and inactivated WA were provided by Nisshin Pharma Inc. (Saitama, Japan). The starch solutions containing WA or inactivated WA were administered using a gastric tube in a volume of 15 mL/kg of body weight.

Blood for glucose, insulin, and triacylglycerol analyses was collected from the tip of tail at time 0 and 0.25, 0.5, 1, 2, and 3 h after starch loading. Plasma glucose was measured by the glucose oxidase method using an assay kit (Glucose E-test; Wako Pure Chemical Industries, Osaka, Japan). Plasma insulin was determined using a highly sensitive rat insulin ELISA kit (Morinaga, Yokohama, Japan). Plasma triacylglycerols were measured using an assay kit containing lipoprotein lipase, glycerol-3-phosphate oxidase, and glycerokinase (Triglyceride E-test; Wako Pure Chemical Industries).

Table 1. Composition of WA Diet

	WA diet			control
	g/kg	kcal/kg	energy %	g/kg
casein	200	800	17.4	200
α-cornstarch	426	1,704	37.0	426
sucrose	50	200	4.3	50
safflower oil	100	900	19.6	100
lard	100	900	19.6	100
cellulose	50			50
AIN^{93} mineral mix	35			35
AIN^{93} vitamin mix	10			10
choline bitartrate	3			3
L-cystine	2			2
WA	24	96	2.1	
inactivated WA				24
total	1000	4600	100	1000

Effects of Feeding Insulin-Resistant Rats a Diet Containing WA. Each experiment consisted of one control rat group and two insulin-resistant rat groups. The control rats were fed the standard laboratory chow (defined as normal rats; n = 5) (see the Supporting Information), and the insulin-resistant rats were fed a diet containing either 2.5% inactivated WA (defined as insulin-resistant rats fed a control diet; n = 6) or 2.5% WA (defined as insulin-resistant rats fed a WA diet; n = 8) (**Table 1**). The rats were fed the diet with either WA or inactivated WA ad libitum for 3 weeks.

After 3 weeks, all animals were fasted for 6 h and killed by decapitation. Mesenteric fat was excised and weighed. All experimental procedures used in the study conformed to the guidelines of the Animal Usage Committee of the University of Shizuoka.

Quantitative RT-PCR. Total RNA was extracted by using the acidified guanidine thiocyanate method as described by Chomczynski and Sacchi (*18*). The total RNA samples were stored at −80 °C for subsequent quantitative RT-PCR analyses. Aliquots of the total RNA samples (0.3 μg) were converted into cDNA by reverse transcription using Super Script III Reverse Transcriptase (Invitrogen, Tokyo, Japan) according to the manufacturer's instructions. To quantitatively estimate the mRNA levels of FAS, acyl-CoA carboxylase (ACC)α, ACCβ, lipoprotein lipase (LPL), acyl-CoA oxidase (ACO), acyl-CoA synthase (ACS), carbohydrate-responsive element-binding protein (ChREBP), sterol regulatory element binding protein (SREBP)1, and β-actin, PCR amplifications were performed using a Light-Cycler instrument system (Roche, Tokyo, Japan). Real-time PCR amplifications were carried out in a total volume of 20 μL containing 400 nM each of gene-specific primers cDNA and SYBR Premix Ex Taq (Takara, Shiga, Japan). The sequences of the PCR primer pairs are shown in **Table 2**. The cycle threshold (CT) values of each gene and β-actin detected by real-time RT-PCR were converted to signal intensities by the delta−delta method (*19*), which calculates the difference of one CT value as a 2-fold difference between the signal for each gene and the signal for a gene for normalization (β-actin). The formula is $[2^{(\text{CT each gene} - \text{CT } \beta\text{-actin})}]$.

Immunoblotting. Total proteins were extracted from the adipose tissue with RIPA buffer [1% NP-40, 0.1% SDS, 0.1% sodium deoxycholate, 20 mM Tris-HCl, pH 8.0, 5 mM EDTA, 150 mM NaCl, protease inhibitor tablet (Roche Molecular Biochemicals, Tokyo, Japan)/10 mL, phosphatase inhibitors (1 mM $NaMoO_4$, 50 mM NaF, and 1 mM Na_3VO_4), 1 mM phenylmethanesulfonyl fluoride]. Lysates were centrifuged at 10000*g* for 10 min at 4 °C. The soluble supernatants were normalized for their total protein concentrations using the Lowry method and stored at −20 °C until analysis. The extracts were separated by 10% SDS-PAGE and transferred to Immobilon membranes (Millipore, Billerica, MA) at 80 V for 90 min in Tris/glycine/methanol transfer buffer. The membranes were blocked for 30 min in 3% bovine serum albumin (BSA) in phosphate-buffered saline (PBS; pH 7.4) containing 0.05% Tween 20 (PBS-Tween) at room temperature. The membranes were then incubated with an anti-FAS antibody (Cell Signaling, Beverly, MA), an antibody detecting both ACCα and ACCβ

Table 2. Sequences of Oligonucleotide Primers Used in This Study

FAS mRNA	5′-GGATGTCAACAAGCCCAAGTA-3′
	5′-TTACAGAGGAGAAGGCCACAA-3′
ACCα mRNA	5′-ATGAACACCCAGAGCATTGTC-3′
	5′-GTGGCCATTCTGAAACTGTGT-3′
ACCβ mRNA	5′-CCCAGGAGGACCCAATAATAA-3′
	5′-CTCTGGAAGTTTGGGGTTTTC-3′
LPL mRNA	5′-TGTCATCGAGAAGATCCGAGT-3′
	5′-CCCGACTTCTTCAGAGACTTGT-3′
DGAT1 mRNA	5′-CTGGCCACAATTATCTGCTTC-3′
	5′-GGAGTATGATGCCAGAGCAAA-3′
ACO mRNA	5′-TCCAGATAATTGGCACCTACG-3′
	5′-GCCACCACTTAATGGAAGTCA-3′
ACS mRNA	5′-GCTGCTTATGGATGACCTCAA-3′
	5′-TCACTGACGTGTTTGCTTGTC-3′
ChREBP mRNA	5′-AATCCCAGCCCCTACACC-3′
	5′-CTGGGAGGAGCCAATGTG-3′
SREBP1 mRNA	5′-ACAAGATTGTGGAGCTCAAG-3′
	5′-TGCGCAAGACAGCAGATTTA-3′
β-actin mRNA	5′-ATGATCTTGATCTTCATGGTGCTA-3′
	5′-GTAAAGACCTCTATGCCAACACAGT-3′

(Cell Signaling), or an antibody against general transcription factor IIB (TFIIB) (Santa Cruz Biotechnology, Santa Cruz, CA) diluted in 3% skim milk in PBS-Tween at 4 °C for >7 h. After a washing with PBS-Tween, the membranes were incubated with biotin-conjugated anti-rabbit IgG (GE Healthcare, Tokyo, Japan) diluted in 3% skim milk in PBS-Tween. After a washing with PBS-Tween and incubation with a horseradish peroxidase-conjugated antibiotin tertiary antibody (Cell Signaling), positive signals were detected by chemiluminescence (ECL Plus; GE Healthcare, Tokyo, Japan) according to the manufacturer's instructions.

Other Assays. Body weight and food intake were measured once every 2 or 3 days. Plasma total cholesterol was measured using an assay kit containing cholesterol esterase and cholesterol oxidase (Cholesterol E-test; Wako Pure Chemical Industries). Plasma nonesterified fatty acid was measured using an assay kit containing acyl-CoA synthase and acyl-CoA oxidase (Nonesterified Fatty Acid C-test; Wako Pure Chemical Industries).

Statistics. Results are expressed as means ± SEM. The significance of differences between two or three groups was determined by using an unpaired Student's *t* test or Dunn's multiple-range test based on the Kruskal−Wallis test, respectively. *p* values of <0.05 were considered to indicate statistical significance.

RESULTS

Effects of a Single Dose of WA on Insulin-Resistant Rats. *Plasma Glucose Concentrations*. As previously described (*17*), the fasting plasma glucose concentration in rats fed the high-fat diet for 11 weeks (124 ± 4.0 mg/dL) was higher ($p < 0.01$) than that in rats fed the control diet (108 ± 2.4 mg/dL). In addition, the plasma glucose (179 ± 5.2 mg/dL) and insulin (6.4 ± 1.1 ng/mL) concentrations at 2 h following OGTT in rats fed the high-fat diet were higher (glucose, $p < 0.01$; insulin, $p < 0.05$) than those in rats fed the standard laboratory chow diet (glucose, 137 ± 7.2 mg/dL; insulin, 2.2 ± 0.8 ng/mL). These results indicate that feeding rats the high-fat diet for 11 weeks induced moderate insulin resistance. To estimate the effects of WA on postprandial hyperglycemia through the inhibition of starch digestion, the plasma glucose concentrations were measured after starch loading. The plasma glucose reached its highest concentration

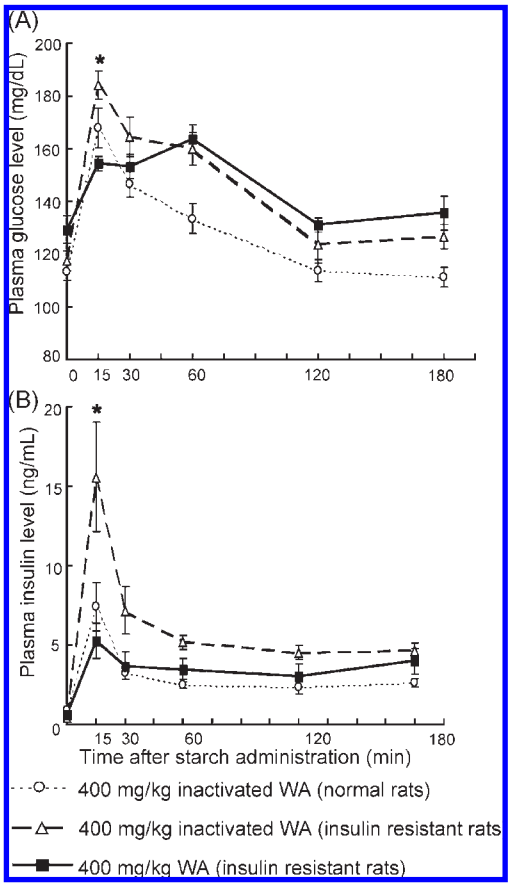

Figure 1. Effects of feeding a diet supplemented with WA on the plasma glucose (**A**) and plasma insulin (**B**) concentrations in starch-loaded insulin-resistant rats. The points and bars indicate means ± SEM (normal rats, $n = 5$; insulin-resistant rats fed a diet containing inactivated WA, $n = 6$; insulin-resistant rats fed a diet containing WA, $n = 8$). ∗, $p < 0.05$, significantly different from insulin-resistant rats fed a diet containing WA (Dunn's test).

(184 mg/dL) at 15 min after starch loading in the insulin-resistant rats treated with inactivated WA and returned to the pretreatment level at 120 min (**Figure 1A**). The plasma glucose concentrations did not differ significantly between the inactivated WA-treated insulin-resistant rats and normal rats. The plasma glucose concentration at 15 min after starch loading was significantly lower in the insulin-resistant rats treated with 400 mg/kg WA compared with those treated with inactivated WA ($p < 0.05$).

Plasma Insulin Concentrations. The plasma insulin reached its peak concentration (15.6 ng/mL) at 15 min after starch loading in insulin-resistant rats treated with inactivated WA and returned to the pretreatment level at 60 min after starch loading (**Figure 1B**). The plasma insulin concentrations were significantly lower in the insulin-resistant rats treated with 400 mg/kg WA than in those treated with inactivated WA at 15 min after starch loading ($p < 0.05$).

Effects of Feeding Insulin-Resistant Rats a Diet Containing WA for 3 Weeks. *Body Weight, Food Intake, and Adipose Tissue Weight*. No differences in body weight, food intake, and dietary energy intake were seen between the insulin-resistant rats fed the WA diet and those fed the control diet (**Table 3**). However, significant differences were observed between normal rats and insulin-resistant rats fed a control diet or WA diet for 3 weeks in their food intake ($p < 0.05$) and dietary energy intake (tendency, normal rats vs insulin-resistant rats fed a control diet; $p < 0.05$, normal rats vs insulin-resistant rats fed a WA diet). No differences in mesenteric adipose tissue weight were seen between any groups

Table 3. Body Weight and Food Intake of Insulin Resistant Rats Fed a Diet Containing WA[a]

		body weight (g)		food intake (g/day)		energy intake (kcal/day)	
animals	test substance	at 0 week	at 3 weeks	at 0 week	at 3 weeks	at 0 week	at 3 weeks
normal rats	chow diet	490 ± 23 a	548 ± 25	22.6 ± 0.6 a	22.3 ± 0.6 a	81.4 ± 4.3 a	80.3 ± 5.0 a
insulin-resistant rats	control	595 ± 26 ab	602 ± 29	9.8 ± 0.6 b	14.5 ± 0.9 b	45.1 ± 2.2 b	66.7 ± 4.6 ab
insulin-resistant rats	WA	593 ± 19 b	590 ± 21	9.1 ± 0.4 b	13.7 ± 0.6 b	41.9 ± 1.8 b	63.0 ± 3.7 b

[a] Values are expressed as means ± SEM for five to eight animals. Values in the same column not sharing a common letter are significantly different from one another at $p < 0.05$ by Dunn's test.

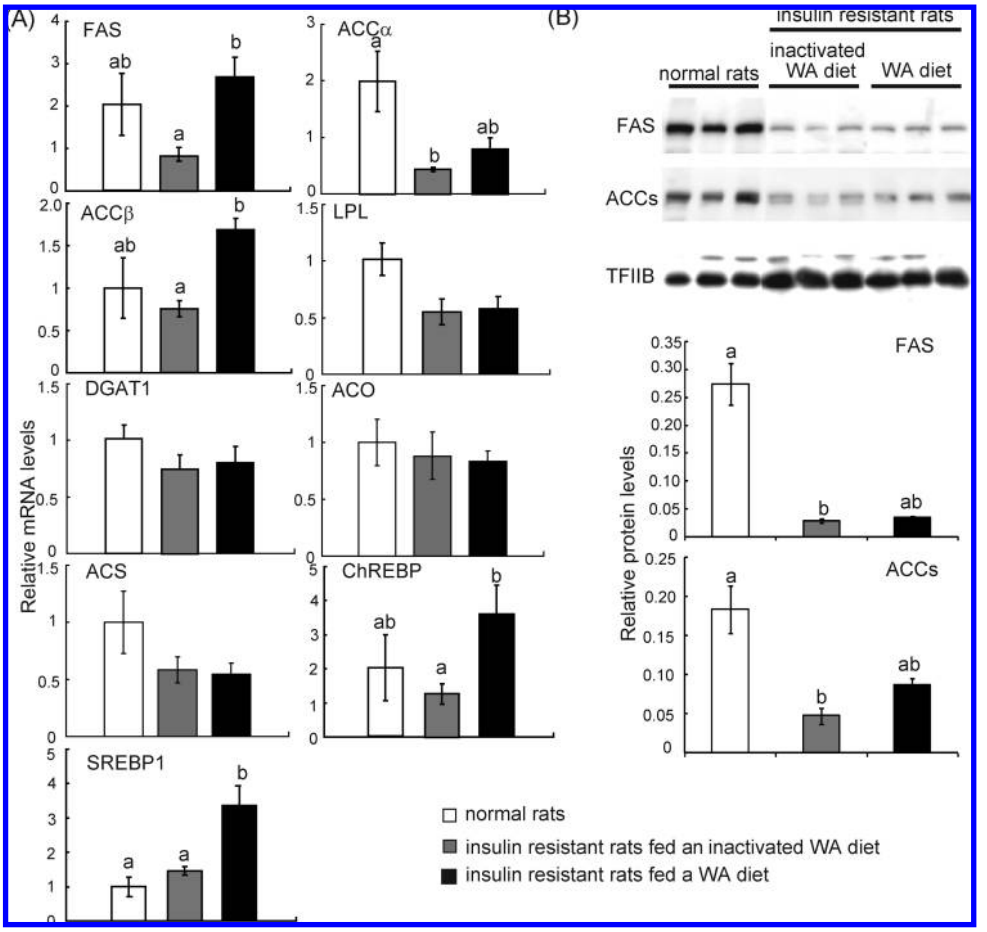

Figure 2. Effects of feeding a diet supplemented with WA on the expression of genes (**A**) and proteins (**B**) related to fatty acid synthesis and their transcriptional factors in adipose tissue of insulin-resistant rats. (**A**) The points and bars indicate means ± SEM (normal rats, $n = 5$; insulin-resistant rats fed a diet containing inactivated WA, $n = 6$; insulin-resistant rats fed a diet containing WA, $n = 8$). (**B**) The data for immunoblotting in typical samples ($n = 3$) is shown in an image; in addition, the graphics are represented. The points and bars indicate means ± SEM (normal rats, $n = 5$; insulin-resistant rats fed a diet containing inactivated WA, $n = 6$; insulin-resistant rats fed a diet containing WA, $n = 8$). Data not sharing common letters differ significantly from each other at $p < 0.05$ (Dunn's test).

of insulin-resistant rats [normal rats, 9.9 ± 0.6 g (a); insulin-resistant rats fed a control diet, 14.9 ± 1.4 g (ab); insulin-resistant rats fed a WA diet, 16.4 ± 0.8 g (b); letter in parentheses denote significant differences from one another at $p < 0.05$ by Dunn's test]. The physical signs in insulin-resistant rats fed a diet containing WA were normal throughout the experimental period.

Effects of Feeding a Diet Containing WA for 3 Weeks on the Expression of Genes Related to Fatty Acid Synthesis and Related Transcriptional Factors in the Adipose Tissue of Insulin-Resistant Rats. To determine whether the inhibition of postprandial hyperglycemia and hyperinsulinemia by feeding the insulin-resistant rats a WA diet altered the expression of genes related to fatty acid synthesis (FAS, ACCα, ACCβ), triacylglycerol accumulation (DGAT1, LPL), β-oxidation (ACO and ACS), or related transcriptional factors involved in fatty acid synthesis (ChREBP and SREBP1), their mRNA levels were measured in the mesenteric fat (**Figure 2**). The mRNA levels of ACCα in the mesenteric fat were significantly lower in insulin-resistant rats fed a control diet than in normal rats. On the other hand, the mRNA levels of FAS, ACCβ, LPL, and ChREBP tended to be lower in insulin-resistant rats fed a control diet than in normal rats. Feeding the insulin-resistant rats a WA diet significantly increased the mRNA levels of FAS, ACCβ, ChREBP, and SREBP1.

Next, we performed immunoblotting for the adipose tissue samples from the three groups using antibodies against FAS and ACCs (detecting both ACCα and ACCβ). The protein levels of FAS and ACCs were significantly lower in insulin-resistant rats fed a control diet than in normal rats. In addition, the protein levels of FAS and ACCs tended to be higher in insulin-resistant rats fed a WA diet than in those fed a control diet (**Figure 2B**).

Table 4. Blood Biochemical Parameters in Insulin Resistant Rats Fed a Diet Containing WA[a]

	normal rats		insulin-resistant rats			
	chow diet		control		WA	
	at 0 week	at 3 weeks	at 0 week	at 3 weeks	at 0 week	at 3 weeks
plasma glucose (mg/dL)	113 ± 3	119 ± 5	117 ± 4	130 ± 7	130 ± 5	130 ± 7
plasma insulin (ng/mL)	0.87 ± 0.33	0.58 ± 0.21	0.43 ± 0.11	0.75 ± 0.4	0.6 ± 0.1	0.33 ± 0.054
plasma triacylglycerol (mg/dL)	113 ± 10	188 ± 23**	106 ± 14	159 ± 23*	128 ± 11	119 ± 7
plasma total cholesterol (mg/dL)	60 ± 6	64 ± 3	74 ± 7	87 ± 4	79 ± 4	81 ± 7
plasma nonesterified fatty acid (mequiv/L)	2.2 ± 0.2	2.8 ± 0.2**	1.3 ± 0.1	1.7 ± 0.2**	1.4 ± 0.1	1.8 ± 0.1**

[a] Values are expressed as means ± SEM for five to eight animals. *$p < 0.05$, **$p < 0.01$; significantly different from 0 week (Student's *t* test).

Table 5. Ratio of Blood Biochemical Parameters at Week 3 to Week 0[a]

	normal rats	insulin-resistant rats	
	chow diet	control	WA
plasma glucose	1.05 ± 0.02	1.11 ± 0.07	1.01 ± 0.1
plasma insulin	0.97 ± 0.17 ab	1.70 ± 0.4 b	0.61 ± 0.12 a
plasma triacylglycerol	1.69 ± 0.19 a	1.51 ± 0.17 ab	0.98 ± 0.09 b
plasma total cholesterol	1.16 ± 0.07	1.22 ± 0.12	1.02 ± 0.1
plasma nonesterified fatty acid	1.33 ± 0.12	1.32 ± 0.14	1.27 ± 0.1

[a] Values are expressed as means ± SEM for five to eight animals. Values in the same column not sharing a common letter are significantly different from one another at $p < 0.05$ by Dunn's test.

Other Assays. The insulin-resistant rats fed a control diet exhibited a significantly greater plasma triacylglycerol concentration (1.5-fold, $p < 0.05$) at 3 weeks after the start of feeding the experimental diet compared with that observed at 0 week. On the other hand, no difference in the plasma triacylglycerol concentrations was seen between weeks 0 and 3 in the WA-treated rats (**Table 4**). The ratio of plasma insulin at week 3 to that at week 0 was higher in the insulin-resistant rats fed a control diet than in those fed a WA diet (**Table 5**).

DISCUSSION

It has already been demonstrated that WA inhibits postprandial hyperglycemia in human subjects (*10*). In the present study, we confirmed that WA inhibited postprandial hyperglycemia in insulin-resistant rats (**Figure 1A**). In addition, we found that WA strongly inhibited the increase in plasma insulin after starch loading (**Figure 1B**). Recent studies have shown that fatty acid synthesis and expression of the related enzyme FAS were decreased in animal models and humans with insulin resistance and that the FAS gene expression levels in animals and humans with insulin resistance were elevated by treatment with a thiazolidine derivative (*2*, *7*). In addition, inhibition of postprandial hyperglycemia is known to improve insulin resistance in human subjects and animal models (*20*, *21*). Considering that insulin resistance leads to higher plasma triacylglycerol, insulin, and glucose concentrations, which are caused by a decreased capacity to accumulate fat in the adipose tissue, it seems likely that the dietary WA supplementation provided to the insulin-resistant rats may have improved the abnormalities in their plasma triacylglycerol, insulin, and glucose concentrations by inducing the expression of genes and proteins related to fatty acid synthesis.

In this study, we found that the plasma triacylglycerol levels were unchanged during the 3 week experimental period in insulin-resistant rats fed the diet supplemented with WA, whereas the insulin-resistant rats fed the control diet exhibited significant increases in their plasma triacylglycerol concentrations. It should be noted that the increase in plasma triacylglycerol by age progression during 3 weeks was greater in normal rats than in insulin-resistant rats fed the control diet. The reason for these observations is considered to be that the dietary energy intake was greater in normal rats than in insulin-resistant rats, because the basal levels of plasma triacylglycerol were similar between the normal rats and insulin-resistant rats. Likewise, the plasma glucose levels tended to increase in the insulin-resistant rats fed the control diet, but did not increase in those fed the WA diet. Furthermore, the ratio of plasma insulin at week 3 to week 0 was lower in insulin-resistant rats fed the WA diet than in those fed the control diet (**Table 5**). These results suggest that dietary WA supplementation to the insulin-resistant rats for 3 weeks improved the hyperinsulinemia and abnormalities of lipid metabolism in plasma.

To examine whether lipid metabolism in adipose tissue is altered by feeding the insulin-resistant rats the WA diet, we performed real-time RT-PCR analyses to determine the mRNA expression levels of genes related to fatty acid synthesis, fat accumulation, and β-oxidation in the mesenteric fat in the insulin-resistant rats fed the WA diet. FAS and ACC are well-known as rate-controlling enzymes for fatty acid synthesis (*22*). LPL is an enzyme required for hydrolysis of triacylglycerol derived from VLDL/chylomicron to fatty acids and glycerol (*23*), and DGAT1 is an enzyme required for resynthesis of triacylglycerol from fatty acids and glycerol in adipose tissue (*24*). ACO (*25*) and ASC (*26*) are rate-controlling enzymes for β-oxidation. The expression levels of many of these genes were lower or tended to be lower in insulin-resistant rats fed the control diet compared with the levels in normal rats. Interestingly, dietary supplementation with WA enhanced the mRNA expression levels of enzymes related to fatty acid synthesis (FAS and ACCβ) in the mesenteric fat of insulin-resistant rats. On the other hand, the expression levels of genes related to the uptake/accumulation of plasma triacylglycerol into adipose tissue, such as LPL and DGAT1, and β-oxidation-related genes, such as ACO and ACS, did not differ between insulin-resistant rats fed the control diet and WA diet. These results indicate that the improvement of insulin resistance in the rats by feeding of the WA diet was caused by the increased expression of genes related to fatty acid synthesis, rather than triacylglycerol accumulation and β-oxidation. In addition, we demonstrated not only that insulin resistance reduced the protein levels of FAS and ACCs in adipose tissue but also that the protein levels of FAS and ACCs tended to be higher in insulin-resistant rats fed the WA diet than in those fed the inactivated WA diet. These findings indicate that the improvements in the plasma triacylglycerol and insulin levels by feeding the WA diet may be caused by up-regulation of FAS and ACCs at the mRNA and protein expression levels in the adipose tissue. Previous studies have demonstrated that fatty acid synthase activity is lower in insulin-resistant rats than in normal rats (*5*, *6*). Because these previous studies also demonstrated that the activity in insulin-resistant rats was up-regulated by improvement of the insulin resistance using pioglitazone and fructo-oligosaccharides, it is considered that the reduced expressions of genes related to

fatty acid synthesis were caused by the insulin resistance. Indeed, WA treatment of insulin-resistant rats improved the plasma triacylglycerol and insulin levels in the present study (**Table 5**). However, dietary energy intake was lower or tended to be lower in insulin-resistant rats than in normal rats (**Table 3**). Therefore, the difference in energy intake between normal rats and insulin-resistant rats may also contribute to the differences in the expression levels of genes and proteins related to fatty acid synthesis. It should be noted that the reductions in FAS and ACCs caused by the induction of insulin resistance were greater at the protein level than at the mRNA level, whereas the inductions of FAS and ACCs in insulin-resistant rats by feeding the WA diet were lower at the protein level than at the mRNA level. These results indicate that the induction of insulin resistance in the rats impaired the protein translation steps for FAS and ACCs as well as the mRNA transcription steps and that WA treatment clearly improved the mRNA transcription steps but had less pronounced effects on the protein translation steps. Future studies should investigate whether long-term treatment of insulin-resistant rats with WA improves the protein translation steps of FAS and ACCs to much greater degrees.

Recently, many studies have demonstrated that the expressions of genes related to fatty acid synthesis are regulated by transcriptional factors such as ChREBP and SREBP1 (*27*−*30*). Indeed, cis-elements for ChREBP and SREBP1 are located in the promoter/enhancer region of the FAS and ACC genes (*28*,*31*,*32*). Therefore, we examined the mRNA levels of these genes in the mesenteric fat of insulin-resistant rats fed the control diet or WA diet. Surprisingly, the mRNA levels of ChREBP and SREBP1 in the insulin-resistant rats were induced by the WA diet (**Figure 2**). It should be noted that SREBP1 has two subtypes known as SREBP1a and SREBP1c. Previous studies have revealed that SREBP1c strongly regulates the transcription of FAS and ACCs (*29*). However, real-time RT-PCR is unable to amplify specific fragments of SREBP1c, although SREBP1a can be detected by a specific primer set. Therefore, the results for SREBP1 in this study represent the total amount of SREBP1. We determined the SREBP1a mRNA expression and found that its level was extremely lower than that for total SREBP1 in the mesenteric adipose tissue (data not shown). Consequently, the total levels of SREBP1 should be affected by changes in SREBP1c rather than SREBP1a. These results indicate that the increased expression of genes related to fatty acid synthesis in the mesenteric adipose tissue induced by feeding insulin-resistant rats the WA diet may be caused by elevations of ChREBP and SREBP1, thereby ameliorating the insulin resistance. Therefore, further studies should investigate whether binding of ChREBP and SREBP1 to the promoter regions of the FAS and ACC genes is increased by feeding insulin-resistant rats a WA diet.

In conclusion, we have demonstrated that the α-amylase inhibitor WA not only ameliorates hyperinsulinemia and lipid abnormalities in the plasma but also induces the expression of genes related to fatty acid synthesis in adipose tissue by inhibiting postprandial hyperglycemia/hyperinsulinemia. Further studies are needed to confirm these findings in other insulin-resistant rat models and to evaluate whether other food components or drugs can reduce postprandial hyperglycemia/hyperinsulinemia.

Supporting Information Available: Diet composition of standard laboratory chow. This material is available free of charge via the Internet at http://pubs.acs.org.

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